

AN INTEGRATED MICROSYSTEM FOR ALLELE-SPECIFIC PCR AMPLIFICATION OF GENOMIC DNA DIRECTLY FROM HUMAN BLOOD

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ABSTRACT

A microsystem for single nucleotide polymorphism detection is demonstrated. The system incorporates two polymerase chain reaction chambers. The first reactor is designed to amplify genomic DNA directly from human blood. PCR amplification from blood can be performed in as little as 11 minutes. The DNA is then purified using an on-chip micropillar filter before entering the second PCR for allele-specific amplification. An electrochemical detector senses if allele-specific PCR amplification has occurred. The influence of template concentration from a first PCR reaction used in the second allele-specific reaction on the detector specificity is assessed.

KEYWORDS: microfluidic, total analysis system, lab on a chip, polymerase chain reaction, single nucleotide polymorphism, genotyping

INTRODUCTION

Single nucleotide polymorphism (SNP) genotyping in the context of personalized medical care has attracted much interest in recent years. Several SNPs are known to have an influence on an individual's predisposition towards an illness or response to a medication. For instance, SNPs in the CYP2C9 gene have important implications in dosing requirements of the anticoagulant drug Warfarin [1]. However, medical care providers are often not equipped to perform SNP genotyping on-site and must ship samples to a specialized lab, which adds cost and time. There is a clear market desire for a point-of-care, microfluidic-based system capable of fast and affordable SNP detection.

Preparing biological samples to extract and purify genomic DNA for further analysis such as SNP detection requires skilled labor and is time consuming. Microsystems with integrated sample preparation have been described in the literature. For instance, Wilding *et al.* [2] describe a system utilizing a filter to capture and purify the genomic DNA-containing white blood cells prior to cell lysis and polymerase chain reaction (PCR). Liu *et al.* [3] utilized magnetic beads for cell capture and purification. The drawback in these approaches is the added size and complexity of the microsystem and increase in time-to-results. In this paper, we report a microsystem capable of DNA amplification directly from human blood without a preliminary wash step utilizing a robust KODfx PCR polymerase. A second allele-specific PCR can then be performed on-chip; afterwards, the sample can be extracted from the microfluidic device and is ready for SNP detection. Furthermore, a microfabricated electrochemical detector has previously been demonstrated to provide fast and sensitive SNP detection [4]. In the future, this sensor can be integrated onto the chip in order to realize a complete microsystem for SNP detection.

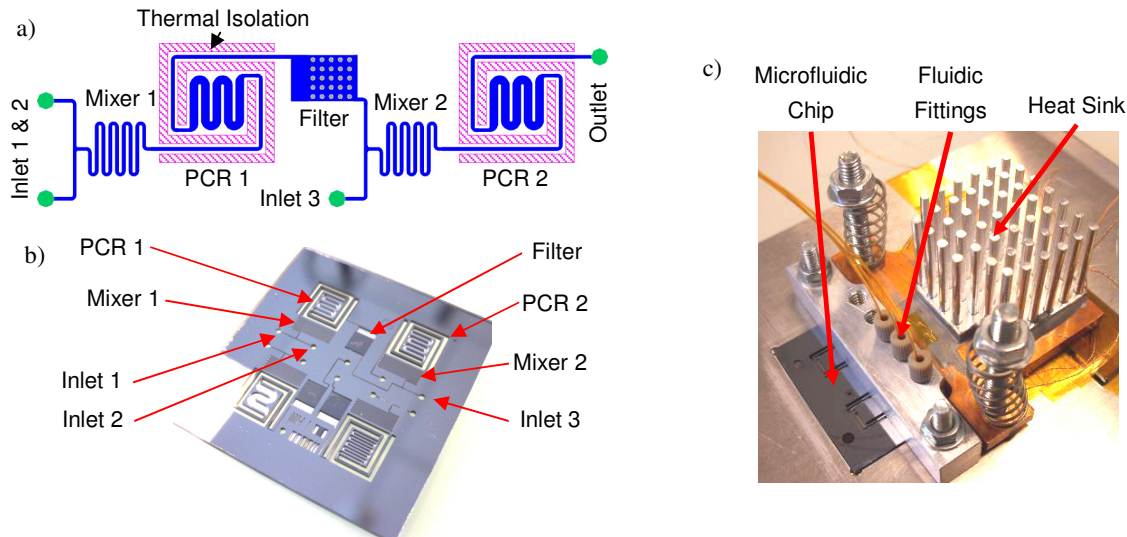


Figure 1: Microsystem for SNP detection: a) overview of microsystem, b) microfabricated silicon and glass microfluidic chip and c) experimental setup for device characterization. All functional components of the microsystem described in this manuscript are in the top half of the chip shown in b). The bottom half of the chip consists of additional test structures.

SYSTEM OVERVIEW

Figure 1a shows the functional layout of the microsystem, while the fabricated silicon-glass based system is shown in Figure 1b. The functional components of the microsystem detailed in this manuscript occupy approximately half of the area of the 3.2 cm by 3.0 cm chip. Blood and PCR reagents are loaded into inlets 1 and 2, respectively, using syringe pumps. After mixing of the two flow streams, the cells are lysed in the first PCR chamber, followed by DNA amplification. The PCR chamber is thermally isolated from the surrounding chip with an air gap etched into the backside of the high thermal conductivity, silicon chip. Thermal design and optimization of the microreactor has previously been reported [5, 6]. After PCR1, the fluid is transported through the filter to remove the cellular remains after lysis. The filtrate containing the amplified DNA is mixed with allele-specific PCR primers from inlet 3. The allele-specific PCR is then performed (PCR2). The sample is extracted from the chip for further analysis.

Figure 1c shows the experimental setup. Microfluidic fittings connect the microsystem to external syringe pumps. A thermal solution has been designed for rapid temperature cycling of the PCR microreactors. The thermal system has previously been detailed elsewhere [6].

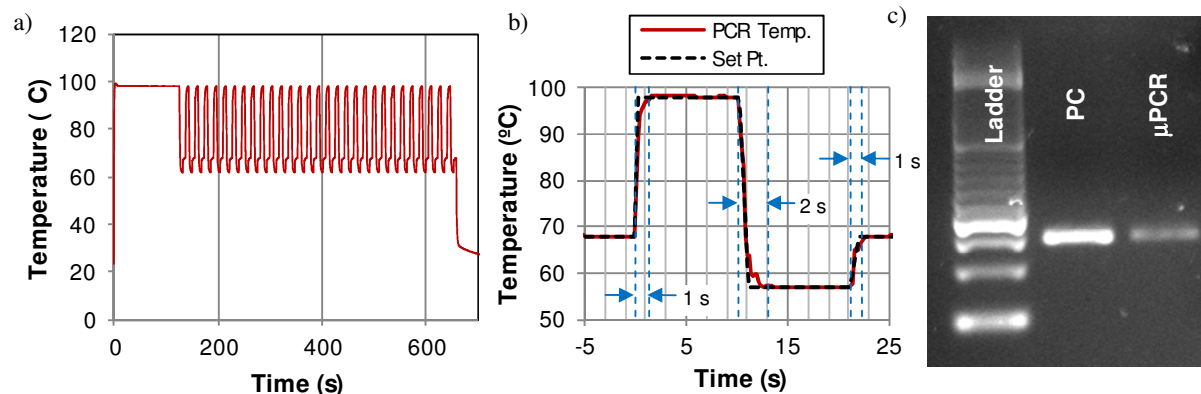


Figure 2: Characterization of PCR microreactor: a) complete thermal cycle, b) single cycle showing ramp rates and c) gel electrophoresis showing amplification of 134 bp genomic DNA fragment direct from blood. PC is positive control (conducted in a commercial PCR tool).

EXPERIMENTS

Figure 2a shows the thermal cycle during PCR. As shown in Figure 2b, total thermal ramp times down to approximately 4 seconds per temperature cycle are achievable, which enables rapid PCR amplification. Successful amplification directly from human blood using the PCR microreactor is also demonstrated (see gel electrophoresis result in Figure 2c). To date, complete cell lysis and PCR thermal cycling can be conducted in times down to 11 minutes.

A filter test device is demonstrated in Figure 3. The material produced from a commercially available PCR tool after cell lysis and PCR amplification is pumped through the filter test device. The cellular remains are removed from the solution using a micropillar array (inter-pillar spacing of 5 μm). Visually, as indicated in Figure 3, the sample is clear after passing through the filter. The DNA in the filtrate can successfully be detected using gel electrophoresis.

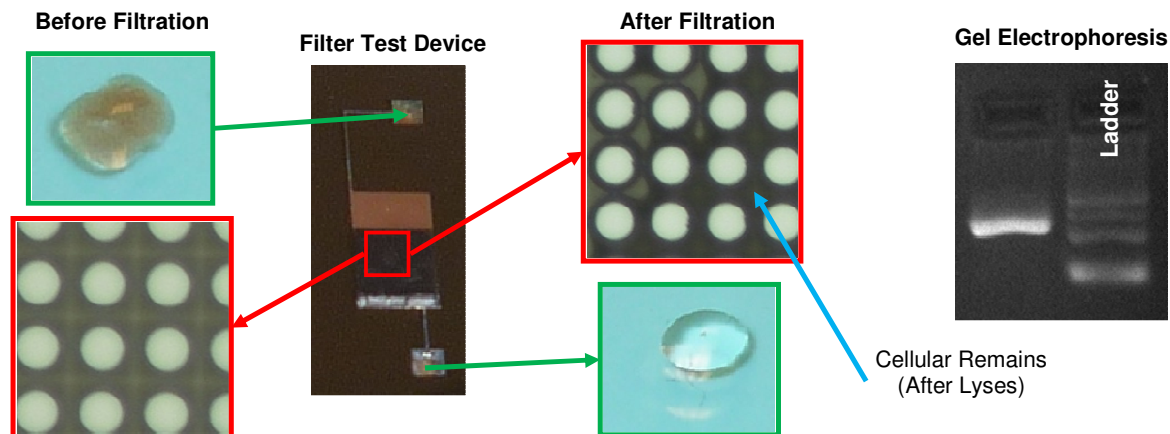


Figure 3: Characterization of micropillar filter for DNA purification. After direct PCR amplification from blood, the sample is passed through a filter test device. Gel electrophoresis of the sample shows successful detection of the amplified DNA product.

The microsystem has a second PCR chamber (PCR2) for performing an allele-specific amplification. By design of the allele-specific primers, PCR amplification only occurs if a SNP is present [7]. To further illustrate the operational concept, allele-specific PCR is conducted in a commercially available tool for determination of AB or O blood type. For this test, the primers are designed so that amplification only occurs in the presence of the AB blood type. Thus, gel electrophoresis shows a positive band for AB and negative for O blood types (see Figure 4a).

An electrochemical sensor is utilized to detect the level of pyrophosphate produced during the allele-specific reaction in PCR2. However, since pyrophosphate is also produced during the first PCR, it is desirable to use only a small portion of the sample from the first reaction as the template for the second reaction. Experiments were conducted to assess the influence of the template concentration used in the second reaction. Results indicate that a good specificity can still be achieved using template concentrations as high as 10%. Since accurate and precise metering of the flow is difficult in application, a higher template concentration is easier to implement on-chip.

Figure 4b shows the electrical measurements from the electrochemical detector. A large current indicates the SNP is present for the AB blood type. Again, the electrochemical measurements show that AB/O determination is reliably achieved with template concentrations as high as 10%.

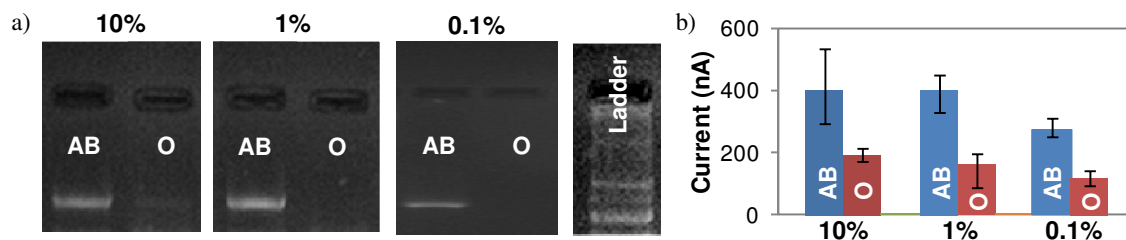


Figure 4: Detection of ABO blood type from a) gel electrophoresis and b) electrochemical detector using three different template concentrations (10%, 1%, and 0.1%).

CONCLUSION

An integrated system for detection of a single nucleotide polymorphism in genomic DNA using allele-specific PCR amplification is detailed. Characterization of the individual system components is described. PCR amplification directly from human blood is demonstrated with times down to 11 minutes. Furthermore, accurate SNP detection using an electrochemical detector is demonstrated even with high template concentrations.

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